Safety and immunogenicity of VPM1002 versus BCG in South African newborn babies: a randomised, phase 2 non-inferiority double-blind controlled trial

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Summary

Background Tuberculosis is a major public health problem worldwide. Immunisation with *Mycobacterium bovis* BCG vaccine is partially effective in infants, reducing the incidence of miliary and tuberculosis meningitis, but is less effective against pulmonary tuberculosis. We aimed to compare safety and immunogenicity of VPM1002—a recombinant BCG vaccine developed to address this gap—with BCG in HIV exposed and HIV unexposed newborn babies.

Methods This double-blind, randomised, active controlled phase 2 study was conducted at four health centres in South Africa. Eligible neonates were aged 12 days or younger with a birthweight of 2·5–4·2 kg, and could be HIV exposed (seropositive mothers) or unexposed (seronegative mothers). Newborn babies were excluded if they had acute or chronic illness, fever, hypothermia, sepsis, cancer, or congenital malformation, or if they received blood products or immunosuppressive therapy. Participants were excluded if their mothers (aged ≥18 years) had active tuberculosis disease, diabetes, a history of immunodeficiency except for HIV, hepatitis B or syphilis seropositivity, received blood products in the preceding 6 months, any acute infectious disease, or any suspected substance abuse. Participants were randomly assigned to VPM1002 or BCG vaccination in a 3:1 ratio, stratified by HIV status using the random number generator function in SAS, using a block size of eight paticipants. The primary outcome was non-inferiority (margin 15%) of VPM1002 to BCG vaccine in terms of incidence of grade 3–4 adverse drug reactions or ipsilateral or generalised lymphadenopathy of 10 mm or greater in diameter by 12 months. The primary outcome was assessed in all vaccinated participants (safety population) at regular follow-up visits until 12 months after vaccination. Secondary immunogenicity outcomes were interferon-γ levels and percentages of multifunctional $CD4^+$ and $CD8^+$ T cells among all lymphocytes across the 12 month study period. The study was registered with ClinicalTrials.gov, NCT02391415.

Findings Between June 4, 2015 and Oct 16, 2017, 416 eligible newborn babies were randomly assigned and received study vaccine. Seven (2%) of 312 participants in the VPM1002 group had a grade 3–4 vaccine-related adverse reaction or lymphadenopathy of 10 mm or greater in diameter compared with 34 (33%) of 104 participants in the BCG group (risk difference –30·45% [95% CI –39·61% to –21·28%]; $p_{non\text{-}inferiority}<0·0001$); VPM1002 was thus non-inferior to BCG for the primary outcome. Incidence of severe injection site reactions was lower with VPM1002 than BCG: scarring occurred in 65 (21%) participants in the VPM1002 group versus 77 (74%) participants in the BCG group (p<0·0001); ulceration occurred in one (<1%) versus 15 (14%; p<0·0001); and abscess formation occurred in five (2%) versus 23 (22%; p<0·0001). Restimulated IFNγ concentrations were lower in the VPM1002 group than the BCG group at week 6, week 12, month 6, and month 12. The percentage of multifunctional $CD4^+$ T cells was higher in the VPM1002 group than the BCG group at day 14 but lower at week 6, week 12, month 6, and month 12. The percentage of multifunctional $CD8^+$ T cells was lower in the VPM1002 group than the BCG group at week 6, week 12, and month 6, but did not differ at other timepoints.

Interpretation VPM1002 was less reactogenic than BCG and was not associated with any serious safety concern. Both vaccines were immunogenic, although responses were higher with the BCG vaccine. VPM1002 is currently being studied for efficacy and safety in a multicentric phase 3 clinical trial in babies in sub-Saharan Africa.

Funding Serum Institute of India.

Introduction

Tuberculosis is one of the widespread infectious diseases worldwide, with about 10 million new cases and 1·5 million deaths in 2020.[1,2] About 1·7 billion people globally are latently infected with *Mycobacterium tuberculosis*.[1]

The *Mycobacterium bovis* BCG vaccine has been used since 1921 with variable efficacy against pulmonary

Research in context

Evidence before this study

We searched PubMed on Dec 31, 2021, for clinical trial reports with the terms "VPM1002", "tuberculosis", "vaccine", and "clinical trial" with no date or language restrictions. We identified two published clinical trials on VPM1002. A phase 1 clinical trial in male volunteers evaluated safety, and immunogenicity of VPM1002 in 80 White adults (60 for VPM1002 and 20 for BCG). This trial reported that VPM1002 was safe and immunogenic for B-cell and T-cell responses. Another phase 2 clinical trial conducted in South Africa in 48 HIV unexposed newborn babies reported that VPM1002 was a safe, well-tolerated, and immunogenic vaccine in newborn babies.

Added value of this study

This study was the first trial to investigate VPM1002 in HIV exposed newborn babies in a high burden tuberculosis setting. Also, this study bridged the hygromycin B resistant formulation of VPM1002 used in all previous studies with a hygromycin B sensitive formulation. VPM1002 was safe and well tolerated in HIV exposed newborn babies. VPM1002 was less reactogenic than the BCG vaccine. Both vaccines were immunogenic, although responses were higher with the BCG vaccine.

Implications of all the available evidence

The BCG vaccine, used widely in newborn babies in multiple low to middle income countries for prevention of tuberculosis, has limitations in terms of safety, especially in immunocompromised infants. BCG also has a variable effect on pulmonary tuberculosis, which is the most common form of tuberculosis. Based on the evidence so far, VPM1002 is being tested in a large phase 3 study to evaluate efficacy in newborn babies for prevention of *M tuberculosis* infection and safety in comparison with the BCG vaccine to achieve marketing authorisation and subsequently to be a possible alternative to the BCG vaccine in immunisation programmes.

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tuberculosis.[3,4] Because BCG has an inadequate effect on the prevention of pulmonary tuberculosis, there is an urgent need to develop more effective tuberculosis vaccines for infants and other at-risk groups.[2] Therefore, a recombinant urease C-deficient listeriolysin (Hly) expressing BCG vaccine strain (VPM1002; Serum Institute of India, Pune, India) was developed as an alternative to the BCG vaccine.[5]

Both major MHC-II restricted CD4+ T-cells and MHC-1 restricted CD8+ T-cells are crucially involved in immunity to *M tuberculosis*.[6–10] BCG strongly induces CD4+ T-cells but only weakly induces CD8+ T-cells.[2,6–12] VPM1002 is a genetic modification of BCG (*M bovis* BCG strain, genetic background Danish, subtype Prague), in which the gene encoding urease C was exchanged for the *Hly* gene of *Listeria monocytogenes*.[5,7,13] The strain, therefore, secretes listeriolysin O into a phagosomal environment with acidic pH for optimal bioactivity,[5,7,13] which enables listeriolysin to perturbate the phagosomal membrane of host cells harbouring VPM1002.[14] Consequently, phagosomal content including proteinaceous and non-proteinaceous compounds from VPM1002 are released into the cytosol.[7] This movement promotes antigen processing through the MHC-I pathway and induces several intracellular alterations including inflammasome activation resulting in elevated IL-1β and IL-18 secretion, apoptosis, and autophagy.[7,10–14] As a result, VPM1002 mimics immune induction by *M tuberculosis*.[7,15] Listeriolysin O is degraded rapidly in the cytosol by the proteasomal compartment through recognition of the PEST sequence preventing harmful consequences of listeriolysin, thereby providing an inbuilt safety mechanism.[7,16]

Initially, VPM1002 (Hyg+) included the hygromycin B resistance gene to enable selection of VPM1002 organisms. Subsequently, the hygromycin B resistance gene was removed.[17] The VPM1002 (Hyg–) strain was comparable to the VPM1002 (Hyg+) strain for quality, efficacy, and safety in animals (unpublished data).

VPM1002 has been tested in three clinical trials: one in Germany in adults[18] and two in South Africa (in adults [NCT01113281] and newborn babies[17]). The phase 1 trial in HIV unexposed adults in Germany and the phase 2 trial in HIV unexposed newborn babies in South Africa showed that VPM1002 induced multifunctional CD4+ and CD8+ T-cell subsets. The interferon γ (IFNγ) release induced by VPM1002 was comparable with BCG.[17,18] Similar findings were seen in the phase 1 trial among adults without HIV in South Africa (unpublished data).

The primary objectives of this study were to compare safety and immunogenicity of VPM1002 with BCG in HIV exposed and HIV unexposed neonates.

Methods

Study design and participants

This was a multicentre, double-blind, randomised, active controlled phase 2 study enrolling healthy newborn babies (aged ≤12 days) from South Africa. The study was approved by the South African Medicines Control Council and the ethics committees from each clinical site (Stellenbosch University Health Research Ethics Committee; University of the Witwatersrand, Johannesburg Human Research Ethics Committee; University of Cape Town Faculty of Health Sciences Human Research Ethics Committee; and Western Cape Government, Strategy and Health, and Tygerberg Hospital). The study adhered to the guidelines of the South African Department of Health and the International Conference on Harmonization of Good Clinical Practice.

Each participant was enrolled only after the mother had given written informed consent. Study enrolment started in June, 2015, and follow-up was completed in October, 2017. The study centres were Tygerberg Academic Hospital (Cape Town), Desmond Tutu Tuberculosis Centre (Cape Town), South African TB Vaccine Initiative (Cape Town), and the Vaccines and Infectious Diseases Analytics Research Unit of the University of the Witwatersrand (Johannesburg). Cape Town and Johannesburg are among the districts with the highest tuberculosis burden in South Africa.[19] The study was registered with ClinicalTrials.gov, NCT02391415.

Eligible babies were aged 12 days or younger with a birthweight of 2·5–4·2 kg. Mothers were aged 18 years or older at screening and were free from active tuberculosis disease and diabetes. For the HIV unexposed group, the mothers were HIV-1 seronegative, and for the HIV exposed group, the mothers were HIV-1 seropositive. Women living with HIV were required to be on antiretroviral therapy (ART) with a screening viral load below 1000 HIV-1 RNA copies per mL.

Newborn babies were excluded in the event of any acute or chronic illness, fever, hypothermia, sepsis, cancer, congenital malformation, or receipt of blood products or immunosuppressive therapy. Mothers with participation in a clinical trial within 3 months before the birth, history of immunodeficiency except for HIV, seropositivity for hepatitis B and syphilis, receipt of blood products in the preceding 6 months, and any acute infectious disease or any reported or suspected substance abuse were excluded.

Randomisation and masking

Participants were randomly allocated (3:1) to VPM1002 or BCG, stratified according to HIV exposure to ensure a 1:1 ratio of HIV exposed and HIV unexposed newborn babies in each group. Participants in the HIV unexposed group allocated to VPM1002 were further randomly assigned to VPM1002 (Hyg+) and VPM1002 (Hyg—) at a 1:2 ratio. VPM1002 (Hyg+) was administered exclusively to HIV unexposed newborn babies. The randomisation schedule was generated using the random number generator function in SAS (version 9.4), using a block size of eight participants.

This was a double-blind study where mothers and caregivers, investigators, and laboratory personnel were blinded to investigational vaccines. Site pharmacists were unblinded and prepared identical syringes for vaccination in the site pharmacy as per the randomisation schedules. Syringes were labelled and given to study team personnel for administration of investigational vaccines to participants, thus maintaining blinding of the study teams.

Procedures

A single dose of VPM1002 or BCG vaccine was administered intradermally on the deltoid aspect of the right arm on day 0. The injection site was covered by an occlusive dressing for 30 min after vaccination. Any direct contact with the vaccination site was avoided to prevent transmission of the vaccine to other parts of the body. VPM1002 (Hyg+) is resistant to hygromycin B, but sensitive to isoniazid, rifampicin, and ethambutol whereas VPM1002 (Hyg–) is sensitive to hygromycin B, in addition to the other drugs.

One vial of VPM1002 was reconstituted with sterile water (1 mL) before injection to make 20 doses of VPM1002. Each dose of 0·05 mL contained $2·5\times10^5$ CFU (range $1–4\times10^5$ CFU). The powder contained excipients such as dextrin and glucose. Batch numbers used were 217H4002 (Hyg–) and 218H4001 (Hyg+).

Initially, BCG manufactured by Statens Serum Institute (Copenhagen, Denmark; Danish strain 151) was used as the reference vaccine but because of an availability issue, BCG manufactured by Serum Institute of India (Pune, India; Moscow strain 361 I) was subsequently used. A single dose of 0·05 mL of both vaccines contains *M bovis* BCG $1–4\times10^5$ CFU. Batch numbers used were 113035A, 114010C, 114007A, 113045A (Statens Serum Institute vaccine), and 037G5282 and 037G5206 (Serum Institute of India vaccine). Study vaccines were transported and stored at 2–8°C.

Post-vaccination follow-up visits were scheduled on day 7 (± 3 days), day 14 (+/– 3 days), week 6 (+/– 4 days), week 12 (+/- 4 days), month 6 (+/- 14 days), and month 12 (+/– 14 days). At month 12, all participants received a tuberculin skin test using the Mantoux method with 2 Tuberculin Units RT-23 (Statens Serum Institute). Mothers and caregivers returned to the clinic with their infants after 48–72 h for assessment of the tuberculin skin test. All infants with a negative tuberculin skin test and no measurable induration were offered the licensed BCG vaccine. Infants with a tuberculin skin test induration of 10 mm or greater were referred for isoniazid preventive therapy as per local standard of care. Safety of the study vaccines was assessed by collecting data for adverse events, local and regional vaccine reactogenicity, physical findings, and laboratory investigations. All adverse events were either observed by the trial investigators, or reported spontaneously by the mother or caregiver, or in a response to a direct question from study personnel. Adverse events were evaluated throughout the study. The injection site and draining lymph nodes of the participant's injection arm were assessed in a standardised manner based on standard clinical judgement by the investigators during follow-up visits. Adverse events were recorded from time of vaccination administration. Adverse drug reactions were regarded as adverse events in which the relationship to the study vaccine was considered by the investigator to be certain, probable, possible, or not assessable. All adverse events were graded for severity on the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (version 2.0), November, 2014, of the US

National Institutes of Health. Adverse events were coded using the preferred term level of the standardised terminology of the Medical Dictionary for Regulatory Activities (version 18.0). Previous and concomitant medicines were coded using the WHO Drug Dictionary (version 2015).

Blood samples for haematology and clinical chemistry were collected on day 14 and week 6. HIV testing by PCR test of all infants from mothers who were either HIV positive at screening or who had seroconverted during the course of the study was performed at week 6 and at months 6 and 12. Additional testing of infants was done if their mothers had seroconverted at week 12. Safety laboratory tests included gamma-glutamyl transferase (GGT), alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, and total bilirubin. Clinically significant abnormal results were reported as adverse events. All safety laboratory tests were performed at the Bio Analytical Research Corporation South Africa, Johannesburg, South Africa.

QuantiFERON-TB-Gold In-Tube test (QFT Gold, Qiagen, Germany) to detect infection with *M tuberculosis* was performed at 6 and 12 months. Participants with positive QFT Gold results were referred for tuberculosis preventive therapy for 6 months, once active disease was excluded.

Venous blood was collected to measure various immunological parameters on day 0, day 14, week 6, week 12, month 6, and month 12. Whole blood was either left unstimulated (negative control) or stimulated with 10 µg/mL purified protein derivative (Tuberculin PPD RT23; Statens Serum Institute) and 1×10^6 CFU/mL BCG culture (Statens Serum Institute), or 5 µg/mL phytohaemagglutinin (HA16; Bioweb, Johannesburg, South Africa; positive control) and incubated for 7 h after which supernatant was collected to measure IFNγ in response to PPD using the ELISA kit ELISAPro (Mabtech, Nacka Strand, Sweden). Brefeldin-A at 10 µg/mL (Sigma, Gillingham, UK) was added for an additional 5 h for flow cytometric analysis to determine the percentage of multifunctional (positive for at least two of IFNγ, tumour necrosis factor [TNF]-α or IL-2), IL17, or GM-CSF positive $CD4^+$ and $CD8^+$ T-cells among all lymphocytes.[20] On average 500 000 CD3 lymphocytes were acquired. Samples for flow cytometric analysis of surface receptors and intracellular cytokines were stained with a panel of antibodies from Becton Dickinson (Germany), acquired on a Becton Dickinson fluorescence-activated cell sorting (FACS) Canto II and analysed using FlowJo software (version 10; Treestar, California, FlowJo LLC) for compensation and analysis. The Becton Dickinson panel included anti-CD3 Pacific Blue (clone UCHT1), anti-CD4 V500 (clone RPA-T4), anti-CD8 APC-Cy7 (clone SK1), anti-GM-CSF PE (clone BVD2-21C11), anti-IFN-γ PE-Cy7 (clone B27), anti-IL-2 PerCP-Cy5·5 (clone MQ1-17H12), anti-IL-17 AlexaFluor 488 (clone N49-653), and anti-TNF-γ APC (clone 6401·1111). Boolean gating was applied to the flow cytometric data to generate combinations of cytokine-expressing $CD4^+$ and $CD8^+$ T-cell subsets responding to stimulation with BCG, PPD, or phytohaemagglutinin (appendix p 4).

See Online for appendix

All immunological tests were performed at the Stellenbosch University Immunology Research Group Laboratory, Stellenbosch, South Africa.

Outcomes

The primary safety outcome was incidence of grade 3 or 4 adverse drug reactions or study vaccine-related ipsilateral or generalised lymphadenopathy of 10 mm or greater in diameter during the 12 month post-vaccination period. The secondary safety outcomes were injection site reactions, unsolicited adverse events, study vaccine-related serious adverse events, abnormal physical examination findings, and abnormal laboratory safety parameters during the 12-month post-vaccination period. The secondary immunogenicity outcomes were concentration of IFNγ in the supernatant of whole blood samples, percentages of multifunctional $CD4^+$ and $CD8^+$ T cells among all lymphocytes, and QFT Gold and Mantoux reaction measured at the final study visit. T-cell assays were performed at baseline, day 14, week 6, week 12, month 6, and month 12. The exploratory safety outcomes were solicited reactions, unsolicited adverse events, and serious adverse drug reactions in HIV unexposed BCG-naive newborn babies vaccinated with a single dose of either VPM1002 (Hyg–) or VPM1002 (Hyg+).

Statistical analysis

The proportion of participants with grade 3 or 4 vaccine-related adverse events or vaccine-related ipsilateral or generalised lymphadenopathy of 10 mm or greater in diameter was computed for each study group. The difference in the proportions between VPM1002 and BCG groups and associated Wald 95% CIs were estimated. VPM1002 was to be considered non-inferior to BCG if the difference between the two groups for the combined incidence of the two primary safety outcomes was below 15%. Non-inferiority of the risk difference was analysed using the Wald test and interpreted at a one-sided 2·5% level.

Assuming no true difference in the incidence of the adverse reactions of interest, and a 20% incidence of these in the BCG group, 396 participants would provide 90% power to support the hypothesis of non-inferiority. Considering a dropout rate of 5% and randomisation in block sizes of eight participants to ensure balance between treatment and HIV exposure groups at each site, 416 participants were enrolled in a 3:1 ratio into the VPM1002 and BCG vaccine groups.

Demographic data for mothers and infants were presented descriptively for each study group. Incidences of all adverse events were calculated and clinically significant abnormal safety laboratory results were summarised for each study group at each timepoint.

Immunological parameters at each timepoint were compared between the study groups using a Wilcoxon rank sum test with continuity correction (for data not normally distributed). The Hodges–Lehmann method was employed in estimating the point difference and 95% CI of the difference in parameters between treatment groups at each visit. Change from baseline for each immunological parameter was analysed per timepoint using a Wilcoxon signed rank test for data not normally distributed. At each timepoint, differences in the median change from baseline between study groups were compared using a Wilcoxon rank sum test (for data not normally distributed). $p<0·05$ was regarded as statistically significant.

Tuberculin skin test reaction using the Mantoux method at study exit was summarised descriptively for each treatment. A χ^2 test was performed to compare distribution of reaction sizes between study groups. The proportion of positive QFT Gold results was calculated at each timepoint for each study group.

The safety analysis population included all participants who were randomly assigned and vaccinated. The immunogenicity analysis population included all participants with complete sets of samples for evaluation of immunological parameters. A balance was maintained among all study groups for the immunological assessments. The per protocol population included all participants in the safety population without any major protocol violations and without household contact with an active tuberculosis patient.

Analysis of demographic, background, and safety data were performed using SAS (version 9.4). Analysis of immunological data was performed using NCSS 2019, version 19.03 and R version 3.5.1, 2018.

Role of the funding source

The funder of the study was involved in the study design, data analysis, data interpretation, and writing of the report.

Results

Between June 4, 2015, and Oct 16, 2017, 727 mothers provided written informed consent, of whom 618 (85%) were screened. 498 newborn babies were screened, with 416 eligible infants randomly assigned to the five study groups (figure). 312 (75%) of 416 newborn babies received VPM1002 and 104 (25%) received BCG

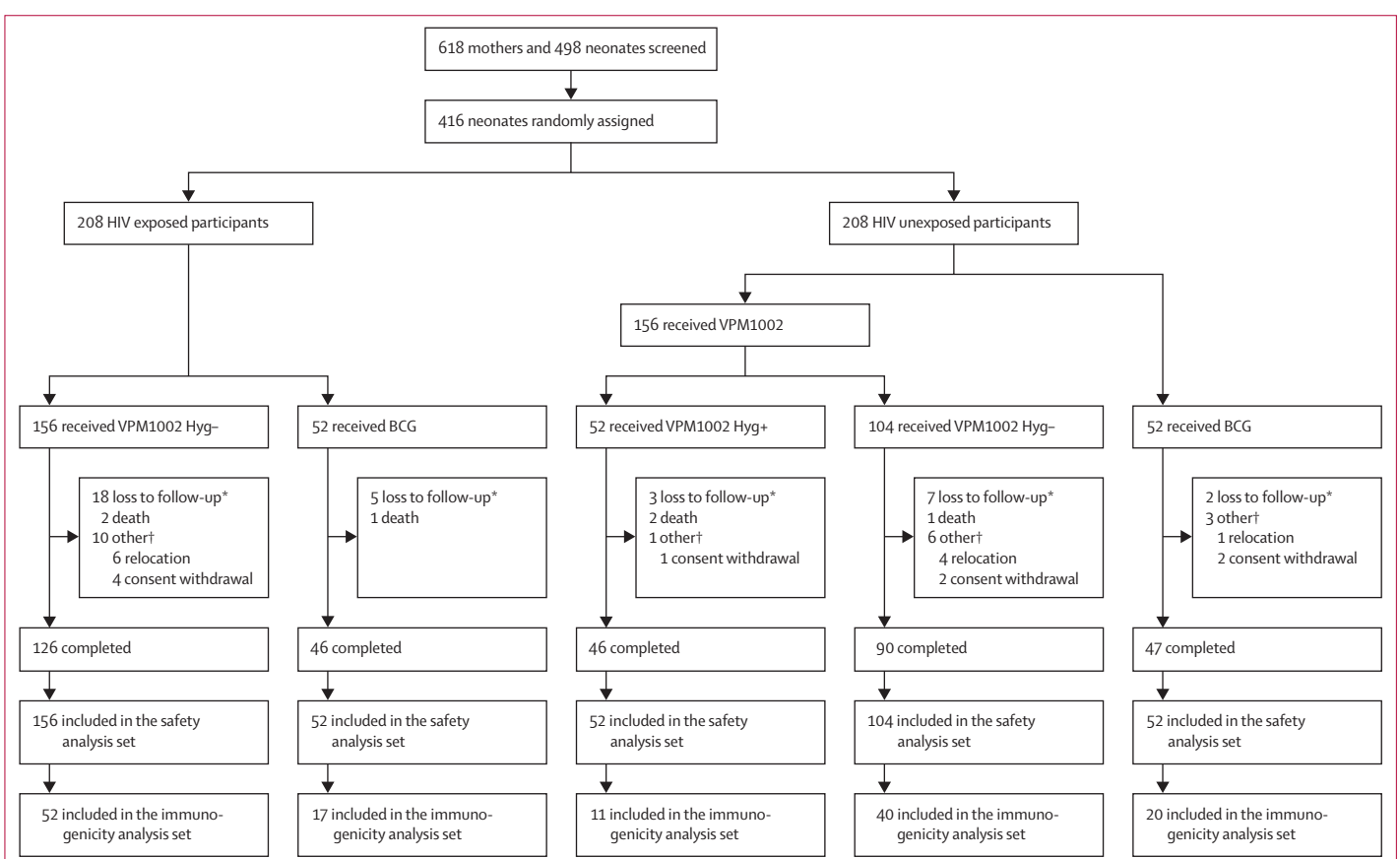

Figure: **Trial profile**

*Even after repeated follow-ups, participants were discontinued from the study as they did not attend the scheduled clinic visits. †Other reasons include either relocation of parents or consent withdrawal by parents.

(73 [17·5%] received BCG Danish and 31 [7·5%] received BCG Moscow). 355 (85%) completed the study and 61 (15%) participants discontinued (none due to adverse events; figure). Retention in BCG-vaccinated participants was 93 (89%) of 104 and 262 (84%) of 312 in the VPM1002-vaccinated group. The primary reason for non-completion in both groups was loss to follow-up (seven [7%] of 104 in the BCG group and 28 (9%) of 312 in the VPM1002 group). All study groups were well balanced for age, birthweight, length, and head circumference; most infants were black African (table 1).

VPM1002 was non-inferior to BCG for incidence of grade 3 or higher adverse reactions, or vaccine-related ipsilateral or generalised lymphadenopathy of 10 mm or greater during the 12 month post-vaccination period (seven [2%] events in 312 participants with VPM1002 *vs* 34 events [33%] in 104 participants with BCG; risk difference −30·45%; 95% CI −39·61% to −21·28%; $p_{non\text{-}inferiority}<0·0001$; table 2).

Adverse events related to infections and infestations, general disorders, and administration site conditions were the most commonly reported in both the BCG and VPM1002 groups. Injection site ulcers and abscesses were more frequent in infants vaccinated with BCG (32 [31%] of 104) than VPM1002 (6 [2%] of 312). Other severe adverse events (grade 3 or higher) were reported with similar frequency in the BCG and VPM1002 groups and were unrelated to study vaccine, apart from one episode of severe somnolence reported in both study groups.

	BCG (n=104)	VPM1002 (n=312)
Age at vaccination (days)	8·7 (2·35)	8·6 (2·10)
Recumbent length (cm)	49·1 (2·51)	49·4 (2·82)
Birthweight (kg)	3·24 (0·347)	3·20 (0·383)
Screening weight (kg)	3·28 (0·404)	3·28 (0·422)
Head circumference (cm)	35·0 (1·52)	34·9 (1·75)
Sex		
Female	48 (46·2%)	146 (46·8%)
Male	56 (53·8%)	166 (53·2%)
Ethnic origin		
Black	82 (78·8%)	244 (78·2%)
Mixed	22 (21·2%)	68 (21·8%)

Data are mean (SD) or n (%).

Table 1: **Baseline characteristics**

Serious adverse events were considered unrelated to vaccination with either BCG or VPM1002.

Pulmonary tuberculosis was reported in one (1%) of 104 infants vaccinated with BCG and in five (2%) of 312 infants vaccinated with VPM1002 (appendix p 6). One HIV exposed infant who received BCG required hospitalisation for pulmonary tuberculosis. All of these cases were diagnosed based on clinical and radiographical grounds by the investigators between 6 months and 12 months after administration of either BCG or VPM1002.

The incidence of vaccine-related adverse events was 97 (93%) of 104 in the BCG group and 247 (79%) of 312 in the VPM1002 group. For HIV exposed infants, 50 (96%) of 52 with BCG and 117 (75%) of 156 with VPM1002 had vaccine-related adverse events. These results were predominantly because the injection site reactions were more common with BCG than VPM1002 (induration 52 [50%] of 104 *vs* 76 [24%] of 312, p<0·0001; injection site scar: 77 [74%] *vs* 65 [21%] p<0·0001; injection site abscess 23 [22%] *vs* five [2%], p<0·0001; injection site ulceration 15 [14%] *vs* one [<1%], p<0·0001; table 3). No notable differences in local reactions were seen between HIV exposed and HIV unexposed infants vaccinated with VPM1002.

Other adverse events considered related to study vaccine included systemic reactogenicity events (ie, crying, somnolence, infantile vomiting, decreased appetite, lymphadenopathy, pyrexia, and rash) and abnormal laboratory results (ie, gamma-glutamyl transferase increased, blood creatinine increased, neutrophil count decreased, haemoglobin decreased, and blood bilirubin increased). The incidences of these events were similar in participants from both the VPM1002 and BCG groups (table 3).

All adverse events, including vaccine-related adverse events, were reported with similar frequency in HIV exposed and HIV unexposed infants receiving VPM1002. The incidence of related adverse events in HIV unexposed infants was 45 (87%) of 52 with the Hyg+ VPM1002 strain and 85 (82%) of 104 with the Hyg– strain (table 3).

The incidence of elevated bilirubin levels at day 14 in HIV unexposed infants was 35 (70%) of 50 for the BCG group and 80 (58%) of 139 for the VPM1002 group, and for those exposed to HIV were three (6%) of 52 in the BCG group and eight (5%) of 148 in the VPM1002 group. For HIV unexposed infants, elevated serum creatinine was

	BCG (n=104)	VPM1002 (n=312)	Difference (95% CI)	$p_{non\text{-}inferiority}$
Grade ≥3 adverse drug reaction	34 (32·7%)	7 (2·2%)	..	..
Investigational medical product-related ipsilateral or generalised lymphadenopathy ≥10 mm	0	0	..	..
Grade ≥3 adverse drug reaction or investigational medical product-related ipsilateral or generalised lymphadenopathy ≥10 mm	34 (32·7%)	7 (2·2%)	−30·45% (−39·61 to −21·28)	<0·0001

Data are n (%).

Table 2: **Primary safety outcomes**

	BCG			VPM1002				
	HIV-u (n=52)	HIV-e (n=52)	All (n=104)	HIV-u (Hyg+) (n=52)	HIV-u (Hyg-) (n=104)	All HIV-u (n=156)	HIV-e (Hyg-; n=156)	All (n=312)
Any adverse reaction	47 (90·4%)	50 (96·2%)	97 (93·3%)	45 (86·5%)	85 (81·7%)	130 (83·3%)	117 (75·0%)	247 (79·2%)
Injection site erythema	21 (40·4%)	17 (32·7%)	38 (36·5%)	26 (50·0%)	40 (38·5%)	66 (42·3%)	48 (30·8%)	114 (36·5%)
Injection site pain	13 (25·0%)	15 (28·8%)	28 (26·9%)	24 (46·2%)	39 (37·5%)	63 (40·4%)	37 (23·7%)	100 (32·1%)
Crying	18 (34·6%)	12 (23·1%)	30 (28·8%)	8 (15·4%)	33 (31·7%)	41 (26·3%)	38 (24·4%)	79 (25·3%)
Injection site induration	26 (50·0%)	26 (50·0%)	52 (50·0%)	7 (13·5%)	26 (25·0%)	33 (21·2%)	43 (27·6%)	76 (24·4%)
Injection site scar	40 (76·9%)	37 (71·2%)	77 (74·0%)	4 (7·7%)	25 (24·0%)	29 (18·6%)	36 (23·1%)	65 (20·8%)
Injection site mass	4 (7·7%)	2 (3·8%)	6 (5·8%)	4 (7·7%)	13 (12·5%)	17 (10·9%)	21 (13·5%)	38 (12·2%)
Somnolence	6 (11·5%)	8 (15·4%)	14 (13·5%)	4 (7·7%)	15 (14·4%)	19 (12·2%)	17 (10·9%)	36 (11·5%)
Injection site swelling	8 (15·4%)	7 (13·5%)	15 (14·4%)	5 (9·6%)	14 (13·5%)	19 (12·2%)	16 (10·3%)	35 (11·2%)
Infantile vomiting	5 (9·6%)	1 (1·9%)	6 (5·8%)	4 (7·7%)	10 (9·6%)	14 (9·0%)	7 (4·5%)	21 (6·7%)
Decreased appetite	1 (1·9%)	3 (5·8%)	4 (3·8%)	1 (1·9%)	7 (6·7%)	8 (5·1%)	7 (4·5%)	15 (4·8%)
Lymphadenopathy	1 (1·9%)	2 (3·8%)	3 (2·9%)	1 (1·9%)	0	1 (1·0%)	11 (7·1%)	12 (3·8%)
Pyrexia	1 (1·9%)	1 (1·9%)	2 (1·9%)	1 (1·9%)	4 (3·8%)	5 (3·2%)	6 (3·8%)	11 (3·5%)
Injection site vesicles	1 (1·9%)	0	1 (1·0%)	1 (1·9%)	3 (2·9%)	4 (2·6%)	3 (1·9%)	7 (2·2%)
Injection site discolouration	0	0	0	2 (3·8%)	4 (3·8%)	6 (3·8%)	1 (1·0%)	7 (2·2%)
Injection site abscess	10 (19·2%)	13 (25·0%)	23 (22·1%)	1 (1·9%)	1 (1·0%)	2 (1·3%)	3 (1·9%)	5 (1·6%)
Injection site erosion	0	0	0	3 (5·8%)	0	3 (1·9%)	2 (1·3%)	5 (1·6%)
Injection site scab	5 (9·6%)	4 (7·7%)	9 (8·7%)	0	0	0	4 (2·6%)	4 (1·3%)
Injection site ulcer	7 (13·5%)	8 (15·4%)	15 (14·4%)	0	0	0	1 (1·0%)	1 (<1%)
Tenderness	0	0	0	0	1 (1·0%)	1 (1·0%)	1 (1·0%)	2 (1·0%)
Rash	0	0	0	1 (1·9%)	0	1 (1·0%)	1 (1·0%)	2 (1·0%)
GGT increased	1 (1·9%)	2 (3·8%)	3 (2·9%)	2 (3·8%)	5 (4·8%)	7 (4·5%)	5 (3·2%)	12 (3·8%)
Blood creatinine increased	0	0	0	0	1 (1·0%)	1 (1·0%)	4 (2·6%)	5 (1·6%)
Neutrophil count decreased	0	0	0	0	0	0	2 (1·3%)	2 (1·0%)
Haemoglobin decreased	0	1 (1·9%)	1 (1·0%)	0	0	0	1 (1%)	1 (<1%)
Blood bilirubin increased	1 (1·9%)	0	1 (1·0%)	0	1 (1·0%)	1 (1·0%)	0	1 (<1%)

Adverse reactions were events assessed as "definitely", "probably", or "possibly" related to the study vaccine or where the relationship was considered to be "not assessable". GGT=gamma-glutamyl transferase. HIV-e=HIV exposed. HIV-u=HIV unexposed. Hyg+=hygromycin resistant. Hyg-=hygromycin sensitive.

Table 3: **Adverse reactions related to study vaccination by MedDRA preferred term**

observed on day 14 in five (10%) of 50 participants in the BCG group and 24 (17%) of 139 participants in the VPM1002 group. For HIV exposed infants, elevated creatinine was noted in 17 (33%) of 52 participants in the BCG group and 49 (33%) of 148 participants in the VPM1002 group.

The median IFNγ concentration in the supernatant as determined by whole-blood ELISA after re-stimulation with PPD was comparable between the two groups at day 0. The median IFNγ concentration was lower in VPM1002 group than the BCG group at week 6 (112·284 pg/mL *vs* 338·893 pg/mL; p=0·0005), week 12 (48·226 *vs* 170·425; p<0·0001), month 6 (14·135 *vs* 35·994; p=0·0033), and month 12 (0 *vs* 29·970; p=0·0008); table 4). Changes from baseline in this parameter were maximal at 6 weeks after vaccination and remained significantly higher than baseline throughout follow up in both the BCG and VPM1002 groups (p≤0.0123; appendix p 7). The magnitude of the increase in IFNγ concentration from baseline was higher in infants vaccinated with BCG than VPM1002 at all timepoints from 6 weeks post-vaccination onwards (p≤0·0016; appendix p 8). VPM1002 elicited a similar immunological response in HIV unexposed and HIV exposed infants (appendix p 7).

Increase in the proportions of multifunctional $CD4^+$ and $CD8^+$ T cells (expressing at least two cytokines of IFN-γ, TNF-α or IL-2) among all lymphocytes after re-stimulation with PPD were seen in response to vaccination with both BCG and VPM1002. The median percentage of multifunctional $CD4^+$ T cells of all lymphocytes was comparable between the groups at day 0, higher in the VPM1002 group (0·022%) compared with the BCG group (0·013%) at day 14 (p=0·0020), but lower in the VPM1002 group compared with the BCG group at week 6 (0·136% *vs* 0·323%; p<0·0001), and subsequent visits (table 5). The median percentage of multifunctional $CD8^+$ T cells of all lymphocytes was also lower in the VPM1002 group compared with the BCG group at week 6 (0·001% vs 0·003%; p=0·043), week 12 (0·001% *vs* 0·002%; p=0·018), and month 6 (0·001% *vs* 0·003%; p=0·011), while it was similar between both groups at day 14 and month 12 (table 5).

Changes observed in the $CD4^+$ T-cell subtype were larger than those observed for $CD8^+$ T cells. The

multifunctional CD4+ T-cell response peaked at 6 weeks post vaccination and remained significantly higher than baseline at all follow-up timepoints in both vaccination groups (p<0·01; appendix pp 5,9). The magnitude of CD4+ T-cell changes was higher for VPM1002 than BCG at day 14 post vaccination (p-value p=0·0041), but higher for BCG compared with VPM1002 at all timepoints from week 6 onwards (p<0·01; appendix p 10). Changes from baseline in multifunctional CD8+ T cells were evident from 6 weeks onward. At 12 months after vaccination, changes from baseline in the proportion of these cells were no longer significant in either BCG or VPM1002 vaccinated infants (appendix pp 5,9). The magnitude of CD8+ T-cell changes were higher in the BCG group than in the VPM1002 group at week 12 and month 6 (p<0·025; appendix p 10). In the VPM1002 group, an increase in the proportion of multifunctional CD4+ and CD8+ T cells after re-stimulation with PPD was seen in both HIV exposed and HIV unexposed groups during the post-vaccination follow-up period (appendix p 11). No significant differences in any of these parameters were detected between HIV exposed and HIV unexposed infants vaccinated with VPM1002 (appendix p 12).

The median proportion of GM-CSF positive CD4+ T cells of all lymphocytes did not differ between the two groups at all timepoints except at week 12 when it was lower in the VPM1002 group than the BCG group (0·008% vs 0·025%; p<0·001; appendix pp 13,14). Changes from baseline in this parameter were significant in both vaccination groups at all timepoints from week 6 post vaccination (p<0·001; appendix p 15). The increase in proportions of GM-CSF positive cells from baseline were higher in the BCG group than the VPM1002 group for week 12 and month 6 (p<0·015; appendix p 16). The proportion of GM-CSF positive CD8+ T cells of all lymphocytes was comparable between the two groups at all timepoints (appendix pp 17,18). Small changes in the proportion of CD8+ T-cells expressing GM-CSF of all lymphocytes, after restimulation with PPD, were evident in both treatment groups post vaccination. Change from baseline was only significant for VPM1002-vaccinated infants at week 6 (p=0·0071) and month 6 (p=0·0363; appendix p 19). There were no significant differences between the two vaccination groups in terms of change from baseline of the proportion of GM-CSF positive CD8+ T cells (appendix p 20). Changes in CD8+ T-cells expressing GM-CSF were less substantial in both treatment groups than the changes observed in CD4+ T cells.

For CD4+ T cells, the proportion of IL-17 positive cells were comparable between both groups at all time points (appendix pp 21–22). Changes from baseline in this parameter were significant only at the week 6 and month 6 post-vaccination timepoints in both vaccination groups (p<0·02; appendix p 23). There were no significant differences in terms of increase in proportions of IL-17 positive cells from baseline between VPM1002 and BCG groups (appendix p 24). The proportions of IL-17 positive CD8+ T cells of all lymphocytes, after re-stimulation with PPD, were higher than those observed for CD4+ T cells, but followed a similar pattern in terms of actual values and changes over time (appendix pp 25–28).

The results of the exploratory immunogenicity analyses obtained after re-stimulation of whole blood samples with PPD were generally supported by similar observations when BCG was utilised as an in vitro stimulant. In addition, exploratory analyses comparing responses with BCG and VPM1002 in HIV exposed infants only, yielded similar results to participants for the entire study cohort (data not shown).

The incidence of positive QFT Gold results was four (4%) of 96 BCG vaccinated infants and nine (3%) of 284 VPM1002 vaccinated infants at 6 months, and two (2%) of 93 BCG vaccinated infants and seven (3%) of 262 VPM1002 vaccinated infants at 12 months. In the HIV exposed infants, the incidence of positive results at

	IFNγ concentrations (pg/mL)		Difference (95% CI)	p value
	BCG	VPM1002		
Day 0				
Participants	37	102	..	..
Mean (SD)	0·55 (2·35)	2·59 (7·97)	..	..
Median	0	0	0 (0–0)	0·2206
Range	0–12·6	0–44·71	..	..
Day 14				
Participants	36	100	..	..
Mean (SD)	4·96 (10·85)	19·84 (34·49)	..	..
Median	0	0	0 (0–3·54)	0·0191
Range	0–53·31	0–191·77	..	..
Week 6				
Participants	37	102	..	..
Mean (SD)	601·40 (684·76)	214·56 (288·41)	..	..
Median	338·89	112·28	−190·02 (−344·23 to −67·79)	0·0005
Range	0–2589·77	0–1829·68	..	..
Week 12				
Participants	37	103	..	..
Mean (SD)	251·44 (272·59)	75·12 (83·75)	..	..
Median	170·43	48·23	−95·62 (−145·55 to −55·43)	<0·0001
Range	0–1150·60	0–341·12	..	..
Month 6				
Participants	37	101	..	..
Mean (SD)	91·81 (150·97)	33·17 (51·84)	..	..
Median	35·99	14·14	−19·71 (−35·99 to −5·44)	0·0033
Range	0–778·98	0–286·07	..	..
Month 12				
Participants	37	103	..	..
Mean (SD)	158·49 (589·69)	17·24 (28·30)	..	..
Median	29·97	0	−15·39 (−31·76 to 0)	0·0008
Range	0–3593·53	0–119·88	..	..

Table 4: **Concentrations of interferon γ in the supernatant as determined by whole-blood ELISA after re-stimulation with tuberculin purified protein derivative for 7 h in VPM1002 and BCG groups**

	Percentage of multifunctional CD4⁺ T cells		Difference (95% CI)	p value	Percentage of multifunctional CD8⁺ T cells		Difference (95% CI)	p value
	BCG	VPM1002			BCG	VPM1002		
Day 0								
n	37	103	..	..	37	103	..	..
Mean (SD)	0·064% (0·2848)	0·024% (0·1148)	..	..	0·004% (0·0142)	0·001% (0·0015)	..	..
Median	0·005%	0·004%	..	..	0%	0%	..	..
Range	0–1·698	0–0·965	0 (–0·002, 0·001)	0·861	0–0·085	0–0·008	0 (0, 0)	0·589
Day 14								
n	37	103	..	..	37	103	..	..
Mean (SD)	0·017% (0·0157)	0·038% (0·1113)	..	..	0·002% (0·0038)	0·003% (0·0107)	..	..
Median	0·013%	0·022%	..	..	0·001%	0·001%	..	..
Range	0–0·070	0–1·140	0·009 (0·004, 0·016)	0·002	0–0·021	0–0·087	0 (0, 0)	0·965
Week 6								
n	37	102	..	..	37	102	..	..
Mean (SD)	0·396% (0·3015)	0·218% (0·5764)	..	..	0·004% (0·0036)	0·007% (0·047)	..	..
Median	0·323%	0·136%	..	..	0·003%	0·001%	..	..
Range	0·027–1·428	0·013–5·860	–0·184 (–0·253, –0·114)	<0·001	0–0·016	0–0·473	–0·001 (–0·002, 0)	0·043
Week 12								
n	36	103	..	..	36	103	..	..
Mean (SD)	0·264% (0·2034)	0·106% (0·0985)	..	..	0·003% (0·005)	0·002% (0·0057)	..	..
Median	0·224%	0·075%	..	..	0·002%	0·001%	..	..
Range	0·057–1·085	0–0·573	–0·132 (–0·171, –0·093)	<0·001	0–0·024	0–0·041	–0·001 (–0·002, 0)	0·018
Month 6								
n	37	102	..	..	37	102	..	..
Mean (SD)	0·133% (0·1239)	0·065% (0·0664)	..	..	0·004% (0·0056)	0·005% (0·027)	..	..
Median	0·102%	0·045%	..	..	0·003%	0·001%	..	..
Range	0·023–0·663	0·007–0·518	–0·048 (–0·068, –0·028)	<0·001	0–0·024	0–0·268	–0·001 (–0·002, 0)	0·011
Month 12								
n	37	103	..	..	37	103	..	..
Mean (SD)	0·13% (0·2351)	0·053% (0·0782)	..	..	0·004% (0·0106)	0·005% (0·0259)	..	..
Median	0·074%	0·031%	..	..	0·001%	0·001%	..	..
Range	0·012–1·367	0–0·581	–0·03 (–0·056, –0·011)	<0·001	0–0·046	0–0·240	0 (0, 0)	0·536

Point estimates and 95% CIs of the difference between the two groups are based on Hodges–Lehmann estimator. p values were analysed by Wilcoxon Rank Sum test with continuity correction.

Table 5: **Percentage of multifunctional CD4⁺ and CD8⁺ T cells of all lymphocytes as determined by whole-blood fluorescence-activated cell sorting-intracellular cytokine staining after re-stimulation with tuberculin purified protein derivative for 12 h in all infants vaccinated with BCG or VPM1002**

6 months was one (1%) of 140 for VPM1002 and three (6%) of 47 for BCG. At month 12, 249 (95%) of 262 infants given VPM1002 had negative results versus 84 (90%) of 93 infants given BCG (appendix p 28).

The overall cumulative incidence of positive QFT Gold results was seven (7%) of 104 BCG vaccinated infants and 14 (4%) of 312 VPM1002 vaccinated infants. In the HIV exposed subset of infants, the incidence of positive QFT Gold results was four (8%) of 52 in the BCG group and four (3%) of 156 in the VPM1002 group (appendix p 28).

At 12 months, more than 70% of participants in both groups had Mantoux reaction size of less than 5 mm. A higher proportion of participants in the BCG group had 5 mm or greater induration than in the VPM1002 group (p=0·0025]; appendix p 29).

Two HIV negative mothers, both of whose infants received VPM1002, seroconverted to HIV during the study. Both infants remained HIV negative during the study. In the HIV exposed groups, one infant from the VPM1002 group became HIV positive at 6 months of age during the study.

Discussion

This study was conducted in HIV exposed and HIV unexposed infants who received either the VPM1002 or BCG vaccine before day 12 of life. VPM1002 appeared less reactogenic than the BCG vaccine. Both vaccines were immunogenic, with the magnitude of BCG-induced immune responses greater than for VPM1002 from week 6 onwards.

The study showed non-inferiority of VPM1002 to the BCG vaccine in terms of the incidence of grade 3 or higher local adverse reactions, or vaccine-related ipsilateral, or generalised lymphadenopathy of 10 mm or greater. This finding is important because local and

regional reactions post vaccination are limitations of the BCG vaccine.[21–25]

VPM1002 was safe in both HIV exposed and HIV unexposed infants. In both of these groups, VPM1002 was associated with a lower incidence of severe injection site reactions such as abscess formation than BCG. VPM1002 might be associated with lower rates of regional and systemic vaccine organism dissemination than BCG, which is of potential benefit to infants with severe acquired or primary congenital immunodeficiencies. WHO recommends that neonates of unknown HIV status born to women living with HIV should be vaccinated if they have no clinical evidence suggestive of HIV infection, regardless of whether the mother is receiving ART.[26] In this study, the incidence of injection site scar was less with VPM1002 (21%) as compared with the BCG (74%; table 3). The relationship between scarring at the injection site and vaccine-induced protection against tuberculosis is not clear. Frankel and colleagues[27] reported that children vaccinated with the Danish BCG vaccine were more likely to develop a scar (97%) versus the Russian BCG (87%; Relative Risk 1·11 [95% CI 1·06–1·16]), but there was no significant effect on health clinic consultations.

WHO recommends that an antimicrobial sensitivity test should be carried out as part of the ongoing characterisation of BCG vaccine strains.[28] The BCG vaccine is required to be sensitive to anti-tuberculosis drugs should treatment be necessary for either local or systemic infection. Although hygromycin B is not used to treat tuberculosis or BCG infection, its resistance was removed from VPM1002 as a matter of abundant caution. Both VPM1002 (Hyg+) and VPM1002 (Hyg–) had similar safety profiles in HIV unexposed infants. These strains were comparable for immunogenicity, thus successfully bridged in this study.

Increased bilirubin was more common in HIV unexposed infants than HIV exposed infants whereas elevated creatinine was more common in HIV exposed infants who received either BCG or VPM1002. The BCG vaccine is not known to cause these abnormalities.[26] Both of these observations could be directly or indirectly attributable to antiretroviral post-exposure prophylaxis in HIV exposed infants. The incidence of neonatal jaundice appears lower in HIV exposed infants than in the HIV unexposed infants, possibly due to the action of nevirapine, a foetal liver enzyme inducer, used to prevent vertical HIV transmission.[29] Nevirapine was also associated with significantly elevated creatinine and urea in juvenile albino rats suggesting that similar effects in human neonates should be assessed.[30] In the phase 2A study of South African infants, increased bilirubin levels were seen in both VPM1002 and BCG groups ($p>0·05$).[17]

VPM1002 and BCG elicited similar immunological responses in HIV unexposed and HIV exposed infants, as assessed by changes in IFNγ concentration in whole blood assays, and cytokine expression in $CD4^+$ and $CD8^+$ T cells. This finding is in contrast to earlier BCG studies which showed lower immune responses in HIV exposed infants than HIV unexposed infants[31,32] although another study had found that T-cell responses to BCG during the first year of life were not altered by HIV-exposure.[33] The IFN-y responses and T-cell responses peaked at week 6, as observed earlier in the phase 2A study.[17]

In this study, restimulation of $CD4^+$ and $CD8^+$ T cells was done with PPD. Specific $CD8^+$ T cells recognise peptide epitopes in the context of MHC 1. MHC 1 loading occurs in the cytosol. Thus, addition of PPD is suboptimal for measuring specific $CD8^+$ T-cell stimulation. Hence, interpretation regarding specific $CD8^+$ T-cell stimulation by BCG versus VPM1002 is difficult. There were only small responses seen for $CD8^+$ T cells in both the groups. Moreover, total $CD8^+$ T-cell populations were analysed and not selected antigen-specific $CD8^+$ T cells, hence it is possible that antigen-specific $CD8^+$ T-cell responses are hidden in the overall response. In addition, BCG restimulation was performed and similar results were observed for the $CD8^+$ T cells.

All immunological parameters were similar between VPM1002 and BCG groups at baseline. In both the groups, these parameters were significantly elevated from baseline after vaccination. Post-vaccination levels of most of these parameters and changes from baseline were higher in the BCG group than the VPM1002 group. The clinical relevance of this finding is unknown. The responses in earlier studies with VPM1002 were comparable to the BCG vaccine in adults and in newborn babies[18] (unpublished data). The frequency and functional profile of BCG-specific $CD4^+$, $CD8^+$, and γδ T-cells from whole blood, including IFNγ producing T-cells, do not correlate with protection against tuberculosis in children.[33,34] For example, although the MVA85A vaccine is a potent inducer of IFNγ, IL-2 ,and TNF, it failed to show efficacy against tuberculosis.[35]

The QFT Gold conversion rate during the 12-month period was 7% in infants vaccinated with BCG and 4% with VPM1002, although the difference was not statistically significant. It remains unknown whether VPM1002 can provide higher protection against *M tuberculosis* infection than BCG as the study was not powered for this objective.

The relatively small sample size was a limitation of this study. We could not statistically power it to compare immunological response to the vaccines. As mentioned earlier, the correlation between T-cell responses and clinical protection has not been completely established.[33,34,35] Also, this study was not powered to assess differences between the two vaccines for QFT Gold and tuberculin skin test responses. To determine antigen-specific responses of $CD8^+$ T cells, more sophisticated assays are needed, including co-culture with antigen presenting cells, and such assays were out of scope of this clinical trial as the allowable blood volumes that can

be drawn from infants is a limiting factor. However, the sample size was adequate to show the primary study hypothesis, which was non-inferiority of the new vaccine against the BCG vaccine in terms of severe adverse reactions. 61 (15%) of 416 participants discontinued the study early, but none due to an adverse reaction. Since the study follow up was 1 year, high loss to follow up was expected, especially in infants. We believe that these missing results do not affect our study findings, especially for safety, given that discontinuations were not caused by adverse events.

Contributors

PSK, LG, SD, DK, SBr, and US contributed to the study design and protocol development. MFC, SAM, AKL, MT, ACH, JS, ES, MH, AK, LJ, AM, and SBh contributed to the data collection. PSK, SD, DK, SBr, LG, HJ, and MFC accessed and verified the data. GW, AG, LAK, DLA, and AGL led the immunogenicity experiments. HJ and MA contributed to the analysis. PSK prepared the first draft of manuscript. DK, SBr, SHEK, and HJ contributed to the preparation of the manuscript. The manuscript was finalised with considerable inputs from all the authors. All authors contributed equally in the conduct of the study, data analysis, and data interpretation. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Declaration of interests

SD, DK, US, and PSK are employed by Serum Institute of India, which manufactures VPM1002. LG and SBr are employed by Vakzine Projekt Management, which developed the VPM1002 vaccine. SHEK and LG are co-inventors and named patent holders for VPM1002.

Data sharing

The data can be accessed from the corresponding author.

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References

1 WHO. Global tuberculosis report 2021. 2021. https://www.who.int/teams/global-tuberculosis-programme/tb-reports/global-tuberculosis-report-2021 (accessed Dec 18, 2021).
2 Andersen P, Scriba TJ. Moving tuberculosis vaccines from theory to practice. *Nat Rev Immunol* 2019; **19:** 550–62.
3 Calmette A, Guérin C, Boquet A, et al. La vaccination préventive contre la tuberculose par le "BCG". Paris: Masson, 1927.
4 WHO. Global tuberculosis control: surveillance, planning, financing: WHO report 2007. 2007. https://apps.who.int/iris/handle/10665/43629 (accessed Aug 31, 2021).
5 Grode L, Seiler P, Baumann S, et al. Increased vaccine efficacy against tuberculosis of recombinant *Mycobacterium bovis* bacille Calmette–Guérin mutants that secrete listeriolysin. *J Clin Invest* 2005; **115:** 2472–79.
6 Scriba TJ, Netea MG, Ginsberg AM. Key recent advances in TB vaccine development and understanding of protective immune responses against *Mycobacterium tuberculosis*. *Semin Immunol* 2020; **50:** 101431.
7 Kaufmann SHE. Vaccination against tuberculosis: revamping BCG by molecular genetics guided by immunology. *Front Immunol* 2020; **11:** 316.
8 Lewinsohn DA, Lewinsohn DM, Scriba TJ. Polyfunctional $CD4^+$ T cells as targets for tuberculosis vaccination. *Front Immunol* 2017; **8:** 1262.
9 Cho S, Mehra V, Thoma-Uszynski S, et al. Antimicrobial activity of MHC class I-restricted $CD8^+$ T cells in human tuberculosis. *Proc Natl Acad Sci USA* 2000; **97:** 12210–15.
10 Winau F, Weber S, Sad S, et al. Apoptotic vesicles crossprime CD8 T cells and protect against tuberculosis. *Immunity* 2006; **24:** 105–17.
11 Schaible UE, Winau F, Sieling PA, et al. Apoptosis facilitates antigen presentation to T lymphocytes through MHC-I and CD1 in tuberculosis. *Nat Med* 2003; **9:** 1039–46.
12 Farinacci M, Weber S, Kaufmann SH. The recombinant tuberculosis vaccine rBCG ΔureC:hly(+) induces apoptotic vesicles for improved priming of CD4(+) and CD8(+) T cells. *Vaccine* 2012; **30:** 7608–14.
13 Hess J, Miko D, Catic A, Lehmensiek V, Russell DG, Kaufmann SH. *Mycobacterium bovis* Bacille Calmette–Guérin strains secreting listeriolysin of Listeria monocytogenes. *Proc Natl Acad Sci USA* 1998; **95:** 5299–304.
14 Saiga H, Nieuwenhuizen N, Gengenbacher M, et al. The recombinant BCG ΔureC:hly vaccine targets the AIM2 inflammasome to induce autophagy and inflammation. *J Infect Dis* 2015; **211:** 1831–41.
15 Wassermann R, Gulen MF, Sala C, et al. Mycobacterium tuberculosis differentially activates cGAS- and inflammasome-dependent intracellular immune responses through ESX-1. *Cell Host Microbe* 2015; **17:** 799–810.
16 Decatur AL, Portnoy DA. A PEST-like sequence in listeriolysin O essential for Listeria monocytogenes pathogenicity. *Science* 2000; **290:** 992–95.
17 Loxton AG, Knaul JK, Grode L, et al. Safety and immunogenicity of the recombinant *Mycobacterium bovis* BCG vaccine VPM1002 in HIV-unexposed newborn infants in South Africa. *Clin Vaccine Immunol* 2017; **24:** e00439–16.
18 Grode L, Ganoza CA, Brohm C, Weiner J 3rd, Eisele B, Kaufmann SH. Safety and immunogenicity of the recombinant BCG vaccine VPM1002 in a phase 1 open-label randomized clinical trial. *Vaccine* 2013; **31:** 1340–48.
19 TBFACTS.org. TB in South Africa - Burden, strategic plan, key populations. 2022. https://tbfacts.org/tb-south-africa (accessed Oct 8, 2021).
20 Hanekom WA, Hughes J, Mavinkurve M, et al. Novel application of a whole blood intracellular cytokine detection assay to quantitate specific T-cell frequency in field studies. *J Immunol Methods* 2004; **291:** 185–95.
21 Sellami K, Amouri M, Kmiha S, et al. Adverse reactions due to the bacillus Calmette–Guerin vaccine: twenty Tunisian cases. *Indian J Dermatol* 2018; **63:** 62–65.
22 Nissen TN, Birk NM, Kjærgaard J, et al. Adverse reactions to the Bacillus Calmette–Guérin (BCG) vaccine in new-born infants—an evaluation of the Danish strain 1331 SSI in a randomized clinical trial. *Vaccine* 2016; **34:** 2477–82.
23 Bolursaz MR, Lotfian F, Velayati AA. Bacillus Calmette–Guérin vaccine complications in Iranian children at a University Hospital. *Allergol Immunopathol* 2017; **45:** 356–61.
24 Venkataraman A, Yusuff M, Liebeschuetz S, Riddell A, Prendergast AJ. Management and outcome of Bacille Calmette–Guérin vaccine adverse reactions. *Vaccine* 2015; **33:** 5470–74.
25 Al-Salem AH, Kothari MR, AlHani HM, Oquaish MM, Khogeer SS, Desouky MS. Safety of intradermal bacillus Calmette–Guerin vaccine for neonates in Eastern Saudi Arabia. *Saudi Med J* 2012; **33:** 172–76.
26 BCG vaccines: WHO position paper – February 2018. *Wkly Epidemiol Rec* 2018; **93:** 73–96. https://apps.who.int/iris/handle/10665/260307 (accessed Aug 31, 2021).
27 Frankel H, Byberg S, Bjerregaard-Andersen M, et al. Different effects of BCG strains—a natural experiment evaluating the impact of the Danish and the Russian BCG strains on morbidity and scar formation in Guinea–Bissau. *Vaccine* 2016; **34:** 4586–93.
28 WHO. Recommendations to assure the quality, safety and efficacy of BCG vaccines, Annex 3, TRS No 979. 2013. https://www.who.int/publications/m/item/trs-979-annex-3-bcg-vax (accessed Aug 31, 2021).

29 Nakanga W, Patel P, Panjwani S, Kennedy N, Kawaza K. Supra-treatment threshold neonatal jaundice: incidence in HIV-exposed compared to non-exposed neonates at Queen Elizabeth Central Hospital in Blantyre, Malawi. *Med J* 2015; **27:** 104–08.

30 Adikwu E, Bonsome B. Renal implication of nevirapine use in juvenile albino rats. *J Interdiscip Histopathol* 2017; **5:** 43–47.

31 Hesseling AC, Jaspan HB, Black GF, Nene N, Walzl G. Immunogenicity of BCG in HIV-exposed and non-exposed infants following routine birth or delayed vaccination. *Int J Tuberc Lung Dis* 2015; **19:** 454–62.

32 Van Rie A, Madhi SA, Heera JR, et al. Gamma interferon production in response to *Mycobacterium bovis* BCG and *Mycobacterium tuberculosis* antigens in infants born to human immunodeficiency virus-infected mothers. *Clin Vaccine Immunol* 2006; **13:** 246–52.

33 Mansoor N, Abel B, Scriba TJ, et al. Significantly skewed memory $CD8^+$ T cell subsets in HIV-1 infected infants during the first year of life. *Clin Immunol* 2009; **130:** 280–89.

34 Kagina BM, Abel B, Scriba TJ, et al. Specific T cell frequency and cytokine expression profile do not correlate with protection against tuberculosis after bacillus Calmette–Guérin vaccination of newborns. *Am J Respir Crit Care Med* 2010; **182:** 1073–79.

35 Tameris MD, Hatherill M, Landry BS, et al. Safety and efficacy of MVA85A, a new tuberculosis vaccine, in infants previously vaccinated with BCG: a randomised, placebo-controlled phase 2b trial. *Lancet* 2013; **381:** 1021–28.